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First record of *Phytomyza rufipes* Meigen, 1830 (Diptera, Agromyzidae) affecting Canola in Uruguay

Ximena Cibils-Stewart[1], Silvina Stewart[1], Alejandro Álvarez[2], Mario Giambiasi[3]

1 *Instituto Nacional de Investigación Agropecuaria, Sistema Agrícola-Ganadero, La Estanzuela, Protección Vegetal, Colonia, Uruguay*
2 *Unión Rural de Flores, Alfredo J. Puig 1179, Flores, Uruguay*
3 *Instituto Nacional de Investigación Agropecuaria, Sistema Vegetal Intensivo, Salto Grande, Biotecnología, Salto, Uruguay*

Corresponding author: *Ximena Cibils-Stewart (xcibils@inia.org.uy)*

Abstract. The leafminer *Phytomyza rufipes* Meigen, 1830 (Diptera, Agromyzidae) is an oligophagous pest of plants of the family Cruciferae. These include Canola, *Brassica napus* L. We report the record of *P. rufipes* in Uruguay, which is confirmed through molecular characterization using DNA barcoding. Characteristic damage symptoms and immature stages of the pest were first confirmed on a Canola crop in Flores, Uruguay.

Key words. mtDNA COI, stem minor, stem borer flies

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INTRODUCTION

Phytomyza rufipes Meigen, 1830 is a leaf-mining fly widely distributed in Europe and a pest of plants in the genus *Brassica* L. (Spencer 1973). It has also been reported in United States and Canada (La Rossa et al. 2005; Scheffer et al. 2008). In South America, it was first reported in Colombia, possibly introduced from Europe (Spencer 1984), and it was later found in Argentina (Valladares et al. 1999).

Phytomyza rufipes (Figure 1) is a leaf- and petiole-miner that feeds exclusively on plants of the cruciferous family, Cruciferae; including Canola (*Brassica napus* L.), Turnip (*Brassica rapa* subsp. *rapa* L.), Cauliflower (*Brassica oleracea* var. *botrytis* L.), Broccoli ((*Brassica oleracea* var. *italica* L.), and a diverse varieties of cabbages (*Brassica oleracea* L.) (Spencer 1973, 1990; Valladares 1984; La Rossa et al. 2005). Adult females deposit their eggs endophytically in the inside of leaves (Valladares et al. 1999). Larvae (Figure 1C, D) mine galleries in the mesophyll or middle layer of the leaf, then through nearest vein they reach the midrib, petiole, and stems where feeding takes place (Figure 2). The amount of damage varies depending on the phenological stage of the crop when the pest attacks, but plants are most significantly damaged when young (Spencer 1973).

Adults are small; males measure around 2.5 mm long and females around 3.5 mm. Adults have yellow head and darker antennas. Legs and pleura are also yellow, as is the fringe scale (Figure 1A, B). Adult frons is unusually wide, three times wider than the eye, and projects outstandingly over the eye on a lateral view (Figure 1B). Female adults occasionally prick leaves with their ovipositors, creating bulges in the underside of the leaves (Figure 2A). The sap that comes out of these pricks is sometimes used as food (Bohm 1957). Females can lay up to 81 eggs during their lifespan (Frey 1951). In Argentina, 0–88 larvae per plant have been observed, with a maximum of 11 per petiole (Valladares et al. 1999). Pupation can occur within the stem or in the soil (Figure 1E, F).

Larvae of this species are whitish and can measure up to 6 mm long. In larvae, posterior spiracles with 25–30 minute pores are distinctive (Figure 1C, D). Larvae mine galleries in the mesophyll of the leaf (Figure 2B, C). Petioles are reached through the veins and are mined in both directions, and, when plants are young, larvae can also mine the stem (Frey 1951; La Rossa et al. 2005). Feeding takes place mainly within the pith of the petiole and stems (Figure 2D–F), and vascular tissue is barely affected. Consequently, leaves often survive even under high pressure from larvae.

Canola can be affected from emergence to maturity (La Rossa et al. 2005), but it is most susceptible in the early vegetative stages. Damage includes discoloration and early leaf loss, which in turn might reduce cold resistance and predispose to lodging (Figure 2G) (La Rossa et al. 2005). Thus, *P. rufipes* nationally

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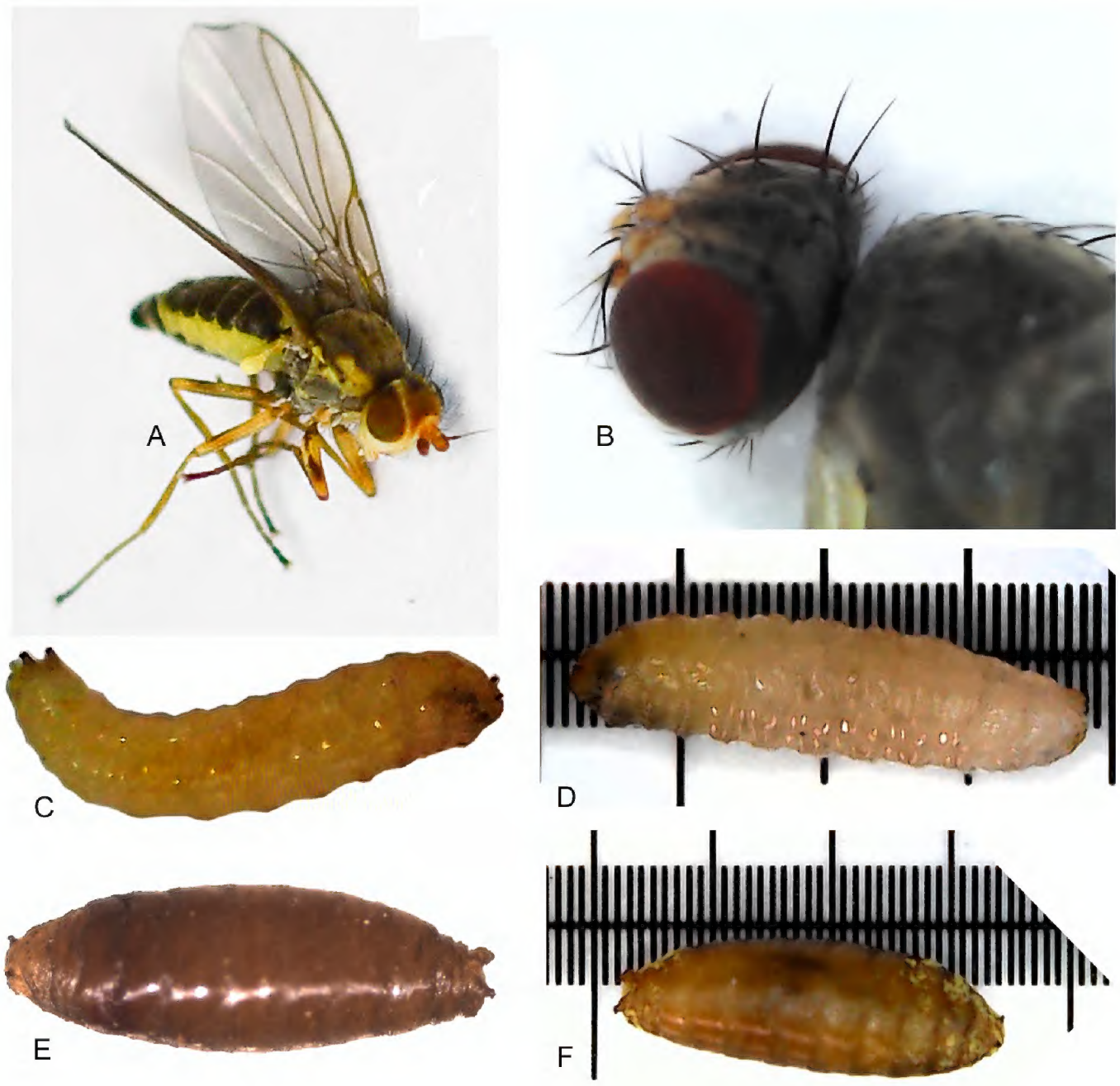


Figure 1. *Phytomyza rufipes* specimens from Uruguay. **A, B.** Adults. **C, D.** Larvae. **E, F.** Pupae. Photo credits: Sebastián Bogliacino (A, C), Ximena Cibils (B, D–F).

represents a high-risk pest due its potential harm to the Canola crop, although yield losses under Uruguayan conditions have not yet been determined.

METHODS

Canola plants with characteristic tunneling on the surface of the stems and pith damage (Figure 2B–F) were found in crop season 2023. The infested stems displayed internal galleries (Figure 2C) or hollow stem (Figure 2D, F), where larvae (Figure 1C, D), pupae (Figure 1E, F), and/or empty puparia were found (Figure 1). Plants were collected in the south-central part of the country, in Flores, and later found in other parts of the country (Figure 3). Stems were placed in cages until adults emerged. The specimens were morphologically identified (Spencer 1973; 1990) and preserved in 95° alcohol for further molecular identification.

Genomic DNA was extracted from individual samples according to a standard CTAB method described by Doyle and Doyle (1987). Entire larvae, pupae, and adult bodies were used individually. A fragment of approximately 658 nucleotides (nt) of the 5′ end of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified by polymerase chain reaction using the primers LepFoIF and COISG-R (Cibils-Stewart et al. 2024). PCR products were purified by the standard method described by Sambrook and Russell (2006) and sent to Macrogen Inc. (Seoul, Korea) sequencing service (http://www.macrogen.com/). The resulting sequences were analyzed using a sequence alignment editor (BioEdit v. 7.2.5; Hall 1999). The taxonomic affinities of our nucleotide sequences were identified using the Basic Local Alignment Search Tool, BLAST (Johnson et al. 2008) and the Barcode of Life Data Systems (Ratnasingham and Hebert 2007). The obtained sequences have been deposited in GenBank with accession numbers PP768979 to PP768985.

The phylogenetic analysis incorporated COI barcode region sequence data from 33 fly specimens. This analysis included 26 nucleotide sequences from GenBank: 17 species of the genera *Phytomyza*, three species *Delia radicum* (Linnaeus, 1758), three *Scaptomyza flava* Fallén, 1823, three *Phytomyza rufipes*, one from Lithuania and two from the United States, and the seven sequences from Uruguay. For this analysis, we employed the UPGMA method. The evolutionary distances were calculated using the Maximum Composite Likelihood method (Tamura et al. 2004) through the MEGA v. 7 software (Kumar et al. 2016).

Figure 2. Damage symptoms associated with *Phytomyza rufipes.* **A.** Oviposition and feeding holes. **B–F.** External and internal stem bore. **G.** Lodged plants due to canola stem borer damage, most likely *P. rufipes.* Picture credits: Sebastián Bogliacino (A), Ximena Cibils (C–F), Alejandro Álvarez (B, G).

RESULTS

Phytomyza rufipes Meigen, 1830

New records. URUGUAY – Flores • Grutas del Palacio; −33.3227, −057.1663; 27.VII.2023; Álvarez leg.; Canola field; 1 adult, 3 puppae, 3 larvae; Genebank: PP768979, PP768980, PP768981, PP768982, PP768983, PP768984, PP768985; INIA code: [La Estanzuela (LE)] LE80, LE82, LE86, LE91, LE96, LE97, LE98.

Identification. The Canola stems under examination displayed distinctive damage patterns indicative of *P. rufipes* infestation, both on the outside of the Canola stem and inside the pith (Figure 2). Furthermore, a close examination of the larvae, with particular emphasis posterior spiracles with 25–30-minute pores revealed a remarkable resemblance to the characteristics of this specific insect species (Spencer 1973, 1984, 1990) (Figure 1C, D). Moreover, the pupal stage, measuring approximately 2.5 mm in size, also presented the distinct feature of well-separated posterior spiracles (Figure 1E, F).

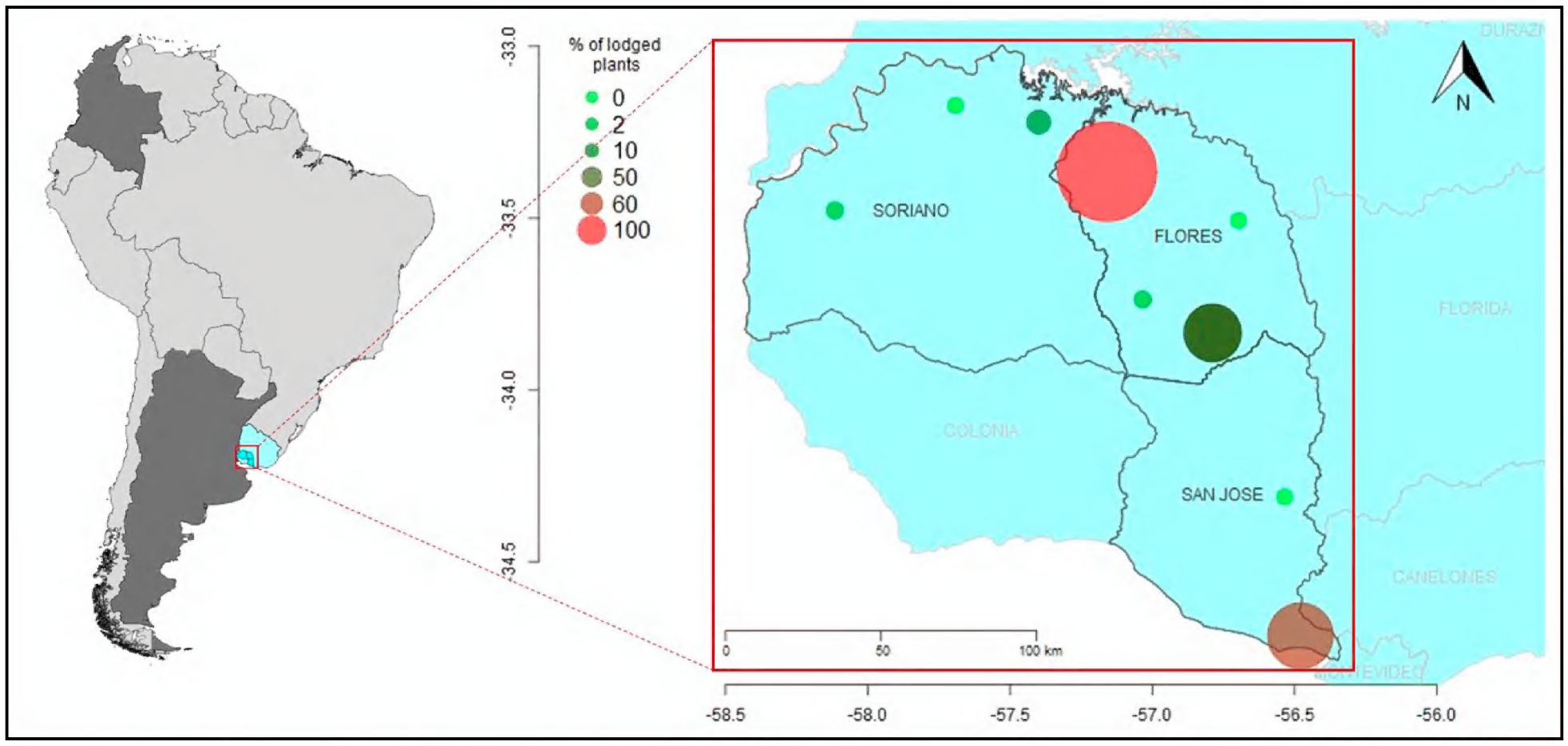
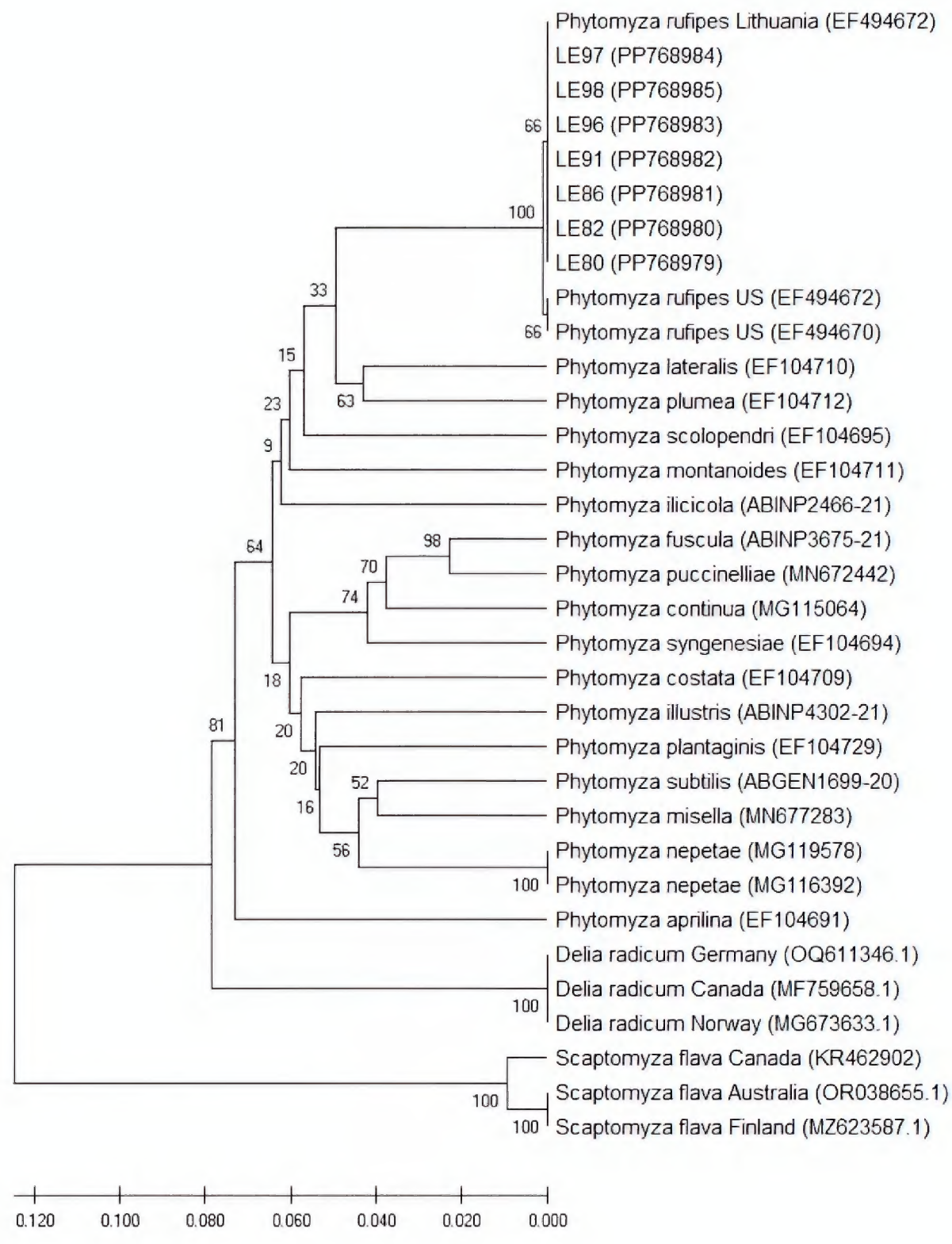


Figure 3. Distribution *Phytomyza rufipes* in South America. Countries in dark gray where *P. rufipes* has already been reported within South America. In light blue, new records of *P. rufipes* in Uruguay with lodged plant percentages (see Figure 2G) at the reported locations. Red circle with 100% lodge plants is the location where the specimens were collected for sequencing.

Figure 4. The evolutionary history was inferred using the UPGMA method. The optimal tree with the sum of branch length (1,15948521) is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004), and they are in the units of the number of base substitutions per site. This analysis involved 33 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). Evolutionary analyses were conducted in MEGA v. 7 (Kumar et al. 2016). This analysis involves 26 nucleotide sequences from GenBank: 17 species of the genera *Phytomyza*, 3 species *Delia radicum* L. (DR), 3 *Scaptomyza flava* F. (SF), and 3 *Phytomyza rufipes* (PR), plus 7 sequences from Uruguay.



In our molecular analysis, the PCR products were successfully sequenced for the DNA barcode region of COI, with a sequence length of 658 bp with no evidence of stop codons or contamination. The sequences obtained were identical to each other. Using the BOLD ID Engine, the comparison with the DNA barcode library resulted in a 100% pairwise nucleotide match over other *P. rufipes* specimens (private) housed in BOLD, confirming their primary identification. NCBI BLAST analysis retrieved a *P. rufipes* nucleotide sequence showing 100% nucleotide identity to our sequences (EF494672.1). Also retrieved were two *P. rufipes* nucleotide sequence showing 98% (EF494671.1 and EF494670.1).

Lastly, the phylogenetic tree (as depicted in Figure 4) reveals that all samples of *P. rufipes* from three different countries clustered independently within a strongly supported clade, as evidenced by robust bootstrap value of up to 100%. In contrast, the various *Phytomyza* species grouped at a higher taxonomic level than the *P. rufipes* clade. Sequences from the *Delia* and *Scaptomyza* genera were positioned in clades that are distant from the *Phytomyza* clade.

DISCUSSION

Canola is a very important crop in Uruguay, and it has increased in area the last few years. In 2022, it reached 35% of the winter-crop area. *Phytomyza rufipes* has some evolutionary adaptations that make it fit to establish in diverse eco-climatic regions, as is, the insertion of eggs into plant tissue that allows neonate larvae to bypass physical defenses and hatch directly into the leaf mesophyll, avoiding desiccation and providing protection from environment and enemies. Additionally, the fact that adults consume leaf exudates from the oviposition wounds provides energy resources in the absence of chewing mouth parts, given adults an extra advantage (La Rossa et al. 2005).

The small size of the adult fly makes it difficult to detect in the field, and consequentially the pest usually goes unnoticed until plants begin to lodge. Chemical control of this fly in Broccoli calabrese, applied over a full six-week period once a week, reduced the damage by about 90% (Coaker 1973); others have reported erratic controls of leaf mining flies due to the larvae's hidden feeding behavior (protected inside the stems) (Curioletti et al. 2018). In Uruguay, there are some registered insecticides for pest management in Canola; however, none of them are registered for the control of insects of the Diptera order.

Altogether about 150 species of Agromyzidae are known to feed regularly on cultivated plants, they normally do not reach high population levels, but occasional outbreaks can occur mainly in species that reproduce rapidly and therefore cause significant yield reduction (Civelek 2002). In Uruguay, crops with the presence of the pest ended up with lodging from 0 to 100% (Figure 3), thus enhanced monitoring in Canola crop to detect *P. rufipes* presence is imperative.

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ADDITIONAL INFORMATION

Conflict of interest
The authors declare that no competing interests exist.

Ethical statement
No ethical statement is reported.

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Author contributions
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Author ORCID iDs
Ximena Cibils-Stewart https://orcid.org/0000-0003-0296-5554
Silvina Stewart https://orcid.org/0000-0003-4472-3422
Mario Giambiasi https://orcid.org/0000-0002-9986-5025

Data availability
All data that support the findings of this study are available in the main text, and all nucleotide sequences used from GenBank are specified in Figure 4.

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